APPENDIX
Showing Changes Made to the Application


In reference to the amendments made herein to the specification and to claims 29, 42, and 45-47, additions appear as underlined text while deletions appear as strikeout text, as indicated below:

**In the Specification:**

At page 40, line 9 through page 42, line 8:

At the 3'-side of the single-stranded nucleic acid thus displaced, there is a sequence F1 complementary to F1c in the same chain. F1 rapidly anneals to F1c in the same molecule to initiate synthesis of a complementary chain. When the 3'-terminal (F1) anneals to F1c in the same chain, a loop containing F2c (i.e., a first loop) is formed. (As illustrated in Fig. 2-(7), the same single-stranded nucleic acid also contains at the 5'-side a sequence R1 complementary to R1c in the same chain, which can likewise anneal to form a loop containing R2, i.e., a second loop.) As is also evident from Fig. 2-(7), the part of this loop containing F2c remains ready for base pairing. The oligonucleotide FA of the invention having a nucleotide sequence complementary to F2c anneals to the part of this loop (i.e., the first loop) and acts as the origin of synthesis of a complementary chain (7). Synthesis of a complementary chain from the loop proceeds while the reaction product in the previously initiated complementary chain synthesis from F1 is displaced. As a result, the complementary chain synthesized with itself as the template is made ready for base pairing again at the 3'-terminal. This 3'-terminal is provided with a region R1 capable of annealing to R1c in the same chain, and the two are annealed preferentially due to the rapid intramolecular reaction (i.e., forming a third loop). The same reaction as the above-described reaction starting from the 3'-terminal synthesized with FA as a template proceeds in this region as well. As a result, the nucleic acid having complementary nucleotide sequences linked alternately in the same single-stranded chain according to the present invention is continued to be extended from R1 as the starting point at the 3'-terminal by successive synthesis of a complementary chain and subsequent displacement thereof. Because R2c is always contained in the loop formed by intramolecular annealing of the 3'-terminal R1, the oligonucleotide (RA) provided with R2 anneals to the loop at the 3'-terminal in the subsequent reaction.

When attention is paid to nucleic acid synthesized as complementary chain from the oligonucleotide annealing to the loop in the single-stranded nucleic acid elongated with itself as the template, synthesis of the nucleic acid having complementary nucleotide

sequences linked alternately in the same single-stranded chain according to the present invention also proceeds here. That is, synthesis of a complementary chain from the loop (i.e., the first loop) is completed when it reached RA in e.g. Fig. 2-(7). Then, when the nucleic acid displaced by this nucleic acid synthesis (i.e., forming the third loop) initiates synthesis of complementary chain (Fig. 3-(8)), the reaction reaches the loop which was once the origin of synthesis (i.e., the first loop), and displacement is initiated again. In this manner, the nucleic acid initiated to be synthesized from the loop is also displaced, and as a result, the 3'-terminal R1 capable of annealing in the same chain is obtained (Fig. 3-(10)). This 3'-terminal R1 anneals to R1c in the same chain to initiate synthesis of complementary chain. This reaction is the same as in Fig. 2-(7) except that F is used in place of R. Accordingly, the structure shown in Fig. 3-(10) can function as a new nucleic acid which continues self-elongation and new nucleic acid formation.

**In the Claims:**

29. (Amended) A method of amplifying a nucleic acid comprising:

A) providing a ~~first~~ template having (i) a 3' end portion comprising a first region located 3' terminal and a first complementary region which, under suitable conditions, anneal to one another to form a first loop, (ii) a 5' end portion comprising a second region located 5' terminal and a second complementary region which, under suitable conditions, anneal to one another to form a second loop, and (iii) a ~~single-stranded target~~ region connecting the 3' end portion and the 5' end portion;

B) extending the 3' terminal of the template to the 5' end of the template by means of a polymerase having strand displacement activity ~~synthesizing a nucleic acid chain complementary to the single-stranded target region using the 3' terminal of the first template~~, when the first region and first complementary region are annealed to one another to form the first loop, ~~as the origin of said synthesizing~~ to form a template extension which includes a third region located 3' terminal and a third complementary region which are substantially the same as the second complementary region and second region, respectively, and which, under suitable conditions, anneal to one another to form a third loop;

C) annealing to the first loop of the ~~first~~ extended template an oligonucleotide primer comprising at the 3' terminal a nucleotide sequence complementary to at least part of the first loop and at the 5' terminal a nucleotide sequence complementary to the first region of the template;

D) extending the oligonucleotide primer along the ~~first~~ extended template, by means of a polymerase having strand displacement activity, to form a ~~second~~ new template complementary to the ~~first~~ template, thereby displacing the ~~first region from the first complementary region and displacing the nucleic acid chain formed during said~~

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~~synthesizing from the first template~~ template extension formed during said extending in step B); ~~and~~

E) further extending the 3' terminal of the extended template to the 5' end of the extended template by means of a polymerase having strand displacement activity, when the third region and the third complementary region are annealed to one another to form the third loop, thereby displacing the new template from the extended template ~~displacing the second template from the first template~~ ; and

F) repeating steps A)-E) using the new template as the template in step A), thereby amplifying the nucleic acid.

42. (Amended) The method according to claim 29 wherein each said ~~synthesizing and said~~ extending ~~are~~ is carried out in the presence of a melting temperature regulator.

45. (Amended) The method according to claim 29, wherein said providing in step A) comprises:

A1) annealing a first oligonucleotide primer to a sample single-stranded nucleic acid molecule ~~comprising the single stranded target region~~, the first oligonucleotide primer comprising a 3' terminal portion which anneals to the sample single-stranded nucleic acid molecule and a 5' terminal portion comprising substantially the same nucleotide sequence as an arbitrary region of the sample single-stranded nucleic acid molecule;

A2) extending the first oligonucleotide primer from its 3' terminal ~~end~~, using a suitable polymerase, to form a first single-stranded nucleic acid molecule comprising (i) a region complementary ~~of the single stranded target region~~ to at least a portion of the sample single-stranded nucleic acid molecule, and (ii) a 5' end portion comprising the 5' terminal portion of the first oligonucleotide primer ~~first region and the first complementary region which, under suitable conditions, anneal to one another to form a loop~~;

A3) displacing the first single-stranded nucleic acid molecule from the sample single-stranded nucleic acid molecule;

A4) annealing a second oligonucleotide primer to the first single-stranded nucleic acid molecule, the second oligonucleotide primer comprising a 3' terminal portion which anneals to the first single-stranded nucleic acid molecule and a 5' terminal portion comprising substantially the same nucleotide sequence as an arbitrary region of the first single-stranded nucleic acid molecule;

A5) extending the second oligonucleotide primer from its 3' end, using a suitable polymerase, to form the ~~first~~ template; and

A6) displacing the ~~first~~ template from the first single-stranded nucleic acid molecule.

46. (Amended) The method according to claim 45, wherein the first oligonucleotide primer used during said ~~first~~ annealing in step A1) or the second oligonucleotide primer used during said annealing in step A4) is the same as the oligonucleotide primer used during said annealing in step C).

47. (Amended) A method of detecting a target nucleotide sequence in a sample comprising:

performing the method of amplifying according to claim 29, wherein the template comprises the target nucleotide sequence, and

determining whether the target nucleotide sequence ~~region~~ is present in the product of the method of amplifying.